

Amendments to the Specification

Please replace paragraph [0009] on page 7 with the following amended paragraph:

[0009] Therefore, one object of the invention is to provide a DNA construct to generate and direct the processing, targeting and stable accumulation of a target protein in transgenic plant seeds. The DNA construct in turn comprises:

- _____a promoter sequence capable of directing expression in plant seed cells;
- _____a first DNA sequence encoding the target protein;
- _____a second DNA sequence having transmembrane domain (TMD) and cytoplasmic tail (CT) sequences serving as anchors for delivering recombinant target proteins via distinct vesicular transport pathways to specific vacuolar compartments; and
- _____a third DNA sequence functioning as a termination region in the plant.

Please replace paragraph [0025] on page 9 with the following amended paragraph:

[0025] Still another object of the invention is to provide a method for constructing a transgenic plant comprising transgenic plant seeds expressing target proteins. The method comprises the steps of:

- _____a) constructing an expression system comprising a vector including and a DNA construct defined herein;
- _____b) transforming plant cells with the vector expression system; and
- _____c) regenerating the transgenic plant from the plant cells to produce the target protein in the plant seeds.

Please replace paragraph [0042] on pages 12-13 with the following amended paragraph:

[0042] Fig. 1 and Fig. 2 show expression cassettes for delivery of soluble proteins to specific PSV subcompartments in transgenic seeds. Chimeric constructs for targeting recombinant proteins to (A) PSV globoid subcompartment via Golgi and (B) PSV crystalloid subcompartment from ER directly (bypassing the Golgi, which would avoid plant Golgi-specific modifications

including N-linked glycosylation). Phaseolin is a seed-specific promoter that allows a high level of expression in transgenic seeds (Altenbach, S.B., *et al.* (1989), Enhancement of the methionine content of seed protein by the expression of a chimeric gene encoding a methionine-rich protein in transgenic plants, *Plant Mol. Biol.* 13, 513–522) and Nos is a 3' terminator. The TMD sequences in (A) and (B) are both derived from BP-80, and the CT sequences in (A) and (B) are derived from BP-80 and α -TIP, respectively (Wink, M. (1993), The plant vacuole: a multifunctional compartment, *J. Exp. Bot.* 44, 231–146; Raikhel, N.V. and Vitale, A. (1999), What do proteins need to reach different vacuoles? *Trends Plant Sci.* 4, 149–155). The coding sequences for the recombinant protein with a signal peptide are cloned in frame between the BamHI and EcoRI sites. The figure is not drawn to scale. Abbreviations and amino acid sequences (underlined sequences being derived from BP-80): CT, cytoplasmic tail sequences from either BP-80 (SEQ ID NO: 1, KYRIRQYMDSEIRAIMAQYMPLDSQEEGPNHV) or α -TIP (SEQ ID NO: 2, KYRIRPIEPPPHHHQPLATEDY); PSV, protein storage vacuole; S, spacer (e.g. SEQ ID NO: 3, DYKDDDDKSKTASQAK or other proteolytic cleavage sequence); sp, signal peptide sequences (e.g. SEQ ID NO: 4, MAHARVLLLALAVLATAAVAVA from proaleurain); TGA; TIP, tonoplast, intrinsic protein; TMD, transmembrane domain sequences from BP-80 (SEQ ID NO: 5, TWAAFVWVVLIALAMIAGGGFLVY).

Please replace paragraph [0047] on pages 15 with the following amended paragraph:

[0047] To illustrate that unique membrane anchors can deliver proteins of different origins to the protein storage vacuoles, we used three proteins as reporters: a yellow fluorescent protein (YFP) that can be detected via auto-fluorescent or anti-GFP (green fluorescent protein) antibody, a hG-CSF (human granulocyte-colony stimulating factor) protein, and a POL (*Polygonatum odoratum* lectin) protein in four expression cassettes. These three proteins were fused at the N-terminal of transmembrane domain (TMD) sequences of BP-80 and the cytoplasmic tail (CT) sequences from either BP-80 (constructs A, C and D) or the alpha-TIP (tonoplast intrinsic protein) (construct B). YFP was fused to constructs A and B, and hG-CSF and POL proteins were fused to

C and D, respectively. In addition, the signal peptide sequences (sp) from the barley cysteine protease aleurain (SEQ ID NO: 6, MAHARVLLLALAVLATAAVAVA) or from the rice storage protein glutelin (SEQ ID NO: 7, MASINRPIVFFTVCLFLLCDGSLA) were included at the N-terminal of the reporter fusion proteins. The resulting fusions were then placed under the control of either the 35S CaMV promoter (constructs A and B) or the seed-specific glutelin Gt1 promoter (constructs C and D) and the Nos 3' terminator. Fig. 3 shows the schematic diagrams of the four expression cassettes constructs used in this invention with information on origins of specific sequences and predicted subcellular localization/pathways. Towards this goal, transgenic tobacco plants expressing construct A or B, and transgenic rice expressing construct C or D have been generated for subsequent analysis of the target proteins expression.

Please replace paragraph [0052] on page 17 with the following amended paragraph:

[0052] We generated transgenic rice expressing construct C (from ~~EXMAPLE-EXAMPLE~~ 1) under the control of the Gt1 seed-specific promoter for further analysis. Similarly, both soluble and membrane proteins were extracted from mature seeds of three individual transgenic plants, followed by SDS-PAGE and Western blot analysis. As shown in Fig. 7, the full-length hG-CSF fusion with a correct expected size was detected only in the membrane fractions of transgenic seeds when anti-hG-CSF antibodies were used (left panel, lanes 2, 4 and 6; double asterisks). Moreover, when another identical set of protein samples was detected using antibodies that recognize the BP-80 CT, the same full-length fusion protein was detected in the membrane fractions of transgenic seeds (right panel, lanes 2, 4 and 6; double asterisks). Again, no such fusion protein was detected in wild type seeds.

Please replace paragraph [0053] on page 17 with the following amended paragraph:

[0053] The system flexibility is further proved by transferring POL fusion (construct D from ~~EXMAPLE-EXAMPLE~~ 1) into rice via *Agrobacterium*-mediated transformation. Mature seeds

obtained from transgenic rice were further analyzed for the expressed proteins. As shown in Fig. 8, the intact POL fusion protein with an expected size was detected in the membrane fractions of transgenic seeds (lanes 4, 5 and 9; double asterisks) when either BP-80 CT or POL antibodies were used in Western blot detection. Again, no signal was detected from wild type seeds (lanes 3, 6, 8 and 10).

Please add the Sequence Listing shown on pages 6-8 of this paper after the Abstract section of the present application. The "Sequence Listing" is also co-filed herewith in computer readable form with a paper copy attached thereto. The Sequence Listing includes seven (7) sequences that are fully supported by the present application as originally filed, see page 12, lines 25-28; page 13, lines 1-2; page 15, paragraph [0047], lines 11-12; claim 9, and no new matter has been added by the amendment.